Distribution of Gastrin in Human, Canine and Cat Digestive Tract Demonstrated by Direct Immunofluorescence

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Distribution of Gastrin in Human, Canine and Cat Digestive Tract Demonstrated by Direct Immunofluorescence

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The existence of gastrin was first reported by Edkins in 1905, and the amino acid sequence was determined in 1964 by Gregory & Tracy, so then it could be synthesized.

Antibody against synthetic human gastrin was successfully produced by McGUIGAN et al (1968) and immunofluorescence and radioimmunoassay were later developed.

The distribution of gastrin in the human digestive organs has been reported by many investigators.

A few questions remain about the distribution of gastrin :

1. Is there gastrin in any other parts of the intestinal tract than the stomach and duodenum?
2. Is there gastrin in the pancreas?
3. Is there gastrin in the salivary gland?
4. Is there a difference of distribution in humans, dogs and cats?

This paper describes a study of the distribution of gastrin in human, dog and cat digestive organs using highly specific immunofluorescence, and a comparison with that in human beings and others.

Material and methods

Human materials :

Stomach tissues were obtained from 15 patients with gastric carcinoma, 15 with peptic ulcer and 4 with benign adenoma or polyp, who were treated with gastrectomy B_{II} retrocolic gastrojejunostomy.

Sections of the antrum were taken just after gastrectomy and tissue level of gastrin was examined by direct immunofluorescence.

The distribution of gastrin in normal stomachs (antrum, body, cardia), duodenum,, jejunum, ileum, colon, rectum, pancreas, parotid gland, and submandibular gland was

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studied by the same method in patients without gastric diseases.

Experimental animals :

Five healthy adult mongrel dogs, weighing about 20 kilograms, and five healthy adult cats weighing about 2-3 kilograms were used in these studies.

The abdomen was opened after satisfactory anesthesia.

Sections of the antrum, body and cardia of the stomach, duodenum, esophagus, jejunum, ileum, colon, pancreas, parotid gland and submandibular gland were taken just after laparotomy.

The direct immunofluorescence technique was applied to formalin fixed and paraffin-embedded tissues.

The antrum and the duodenum were examined at 2cm intervals.

Immunofluorescence :

Direct immunofluorescent technique was applied to formalin fixed, paraffin embedded tissues.

Specific antiserum to synthetic human gastrin I was obtained by a modification of McGUIGAN's method. The IgG fraction of this antiserum was separated and labelled with FITC by following procedure.

Preparation of antigen :

Synthetic human gastrin I (Imperial Chemical Industries Ltd.) was coupled to bovine serum albumin (Sigma Chemicals Comp.) by a modification of McGUIGAN's method[11]. Two mg of bovine serum albumin was dissolved in 2 ml of 0.1 M potassium phosphate buffer, pH 7.4, containing 1 ml of N, N-dimethylformamide. To this solution 2mg of synthetic human gastrin was added. After cooling to 0°C, 2mg of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide was added three times at intervals of 30 minutes.

If necessary, the pH was adjusted to 7.4 with N/100 hydrochloric acid, then the mixture was allowed to stand overnight at 0°C. To this reactive mixture 5 mg of carbodiimide was added again and stirred at room temperature for 4 hours, after which it was dialyzed at 4°C for 24 hours against 5 L of M/100 potassium phosphate buffer containing 0.15M sodium chloride. The resultant slightly opalescent solution was dialyzed against 5 L of distilled water at 4°C for 12 hours twice. Following dialysis, it yielded 3.2 mg after lyophilization.

Immunization of Rabbit :

Two mg of SHG I-bovine serum ablumin conjugate in phosphate buffer solution (pH 7.2) was mixed well with complete adjuvant (1cc) until it completely emulsified.

This emulsion was injected into the foot pad.

Two months later SHG-bovine serum albumin cojugate (1mg) in phosphate buffer solution was injected intravenously as a booster.

Ten days after this booster injection a small amount of serum was evaluated by radioimmunoassay and the Ouchterlony agar gel double diffusion test. The latter test showed definite evidence of specific antibody to SHG I (Fig. 1), so whole blood was drawn by cardiac puncture.

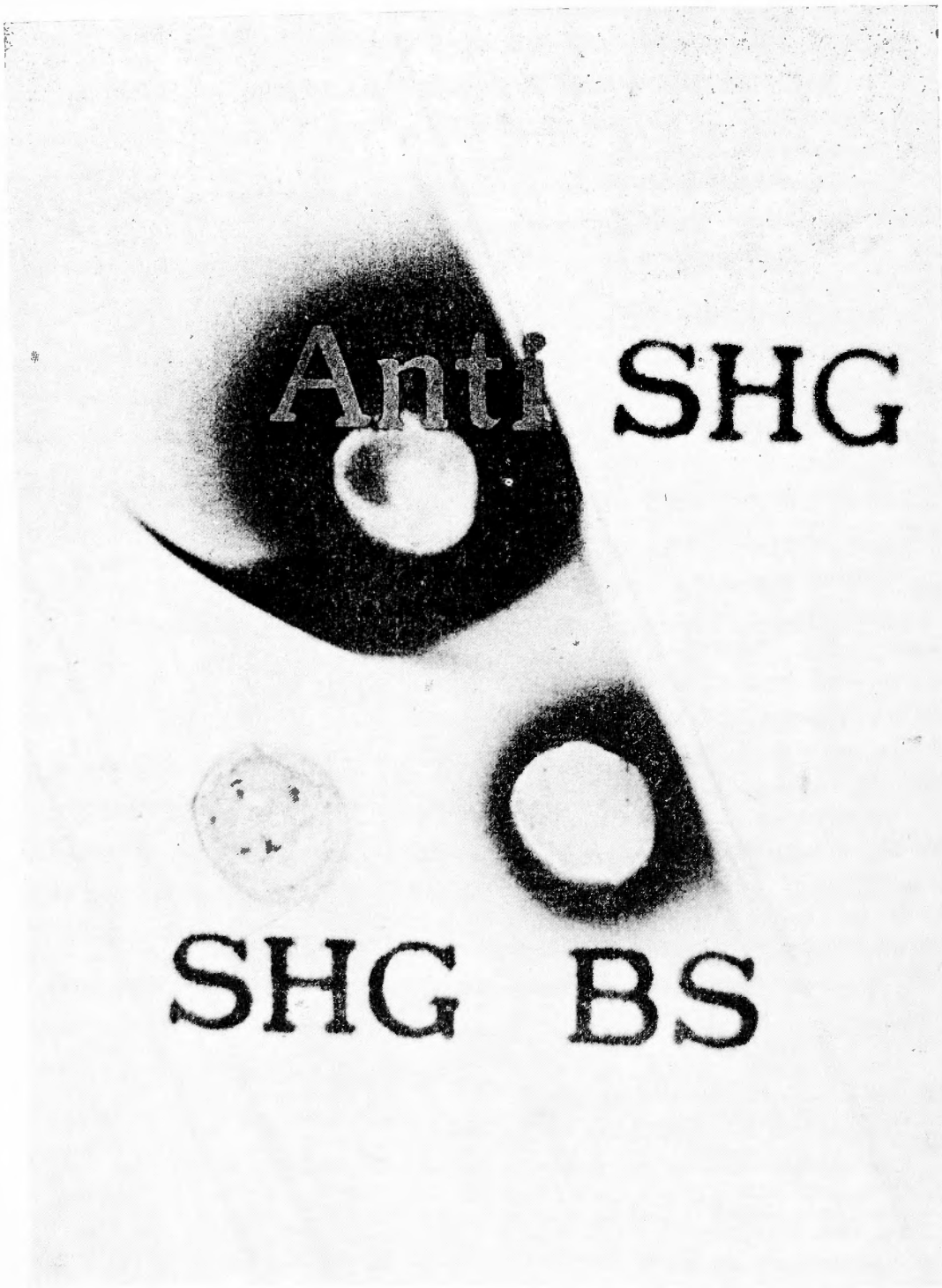

Fig. 1. Ouchterlony agar gel double diffusion test of anti-serum to synthetic human gastrin I (SHG). The test shows definite evidence of specific anti-body to SHG.

Preparation of FITC labeled anti-gastrin antibody :

Anti-serum to Gastrin (SGH 1) was precipitated at 4°C by dropwise addition of saturated ammonium sulfate. Repeatedly dyalized precipitate in buffer was conjugated with FITC (Baltimore Biological Laboratories) at 4°C for 6 hours during constant stirring with a magnetic stirrer. After dialysis, it was applied to a DEAE cellulose column, eluted with 0.05M phosphate buffer (pH 6.3) and collected as fraction No. 1.

Its protein concentration was 4.8mg/ml and its FP ratio was 1.4.

Direct immunofluorescent technique :

Antral mucosa or normal portions of various tissues were taken immediately after surgery, and small pieces were fixed for 12 hours in cold 10% formaldehyde and embedded in paraffin.

Paraffin embedded blocks were sliced and after deparaffinization the sections were washed well with cold staining buffer solution (pH 7.2).

Specific anti-Gastrin antibody contaning globulin labelled with FITC obtained by the above method was layered over the sections for 12 hours. After thorough washing with cold staining buffer solution, the sections were covered with a cover glass and studied by fluorescent microscopy (Carl Zeiss). The specificity of the staining was examined by the blocking test with SHG solution.

Results

Distribution of gastrin in the human degestive tract.

Stomach :

Gastrin-containing cells were numerous in the antrum of the human stomach (Fig. 2) as McGUIGAN et al[12)13)18)] already noted. They were situated in the middle zone of the antral mucosa.

They were most heavily concentrated in the middle portion (2-4cm proximal to the pylorus) of the antrum and became sparser in the pylorus and beyond. They were pyramidal, the typical shape of endocrine cells in the GEP system.

In the cardia or the body of the stomach there were no gaistrin-containing cells.

Duodenum :

In the human duodenum gastrin-containing cells could be found, as shown Fig. 3 or 4. In the duodenal bulbus there was sparse G-cell zone near the pylorus.

The number of gastrin-containing cells in the human duodenum is definitely lower than in the antrum, and it becomes increasingly smaller with distance from the pylorus.

In the 3rd portion of the duodenum the population was very scant (Fig. 4).

Esophagus, Jejunum, Ileum, Colon and Rectum :

No gastrin-containing cells could be identified in the esophagus, jejunum, iluem, colon or rectum (Fig. 5, 6).

Pancreas, Parotid gland and Submandibular gland :

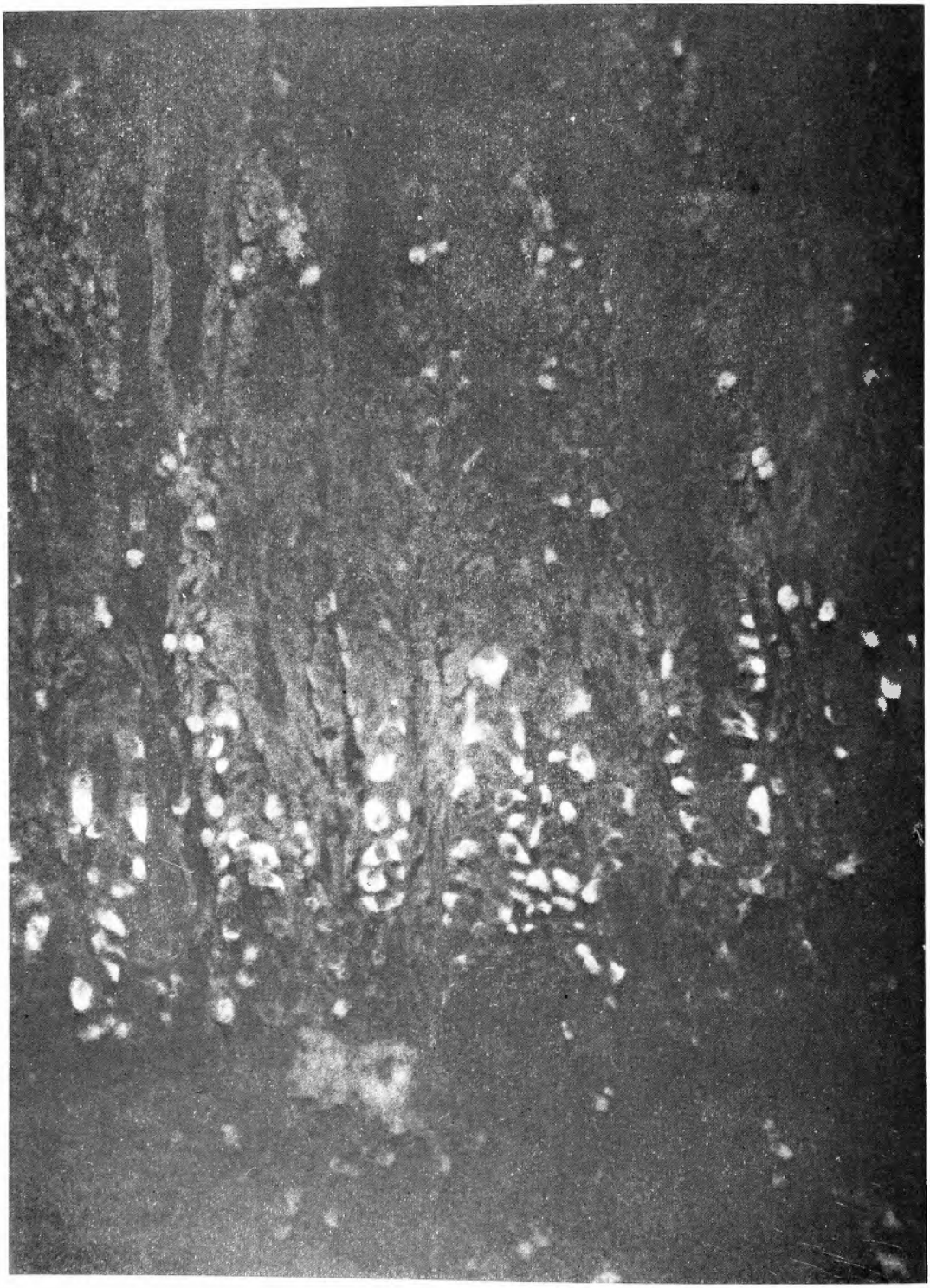

Fig. 2. Gastrin-containing cells in the antrum of the human stomach. (× 160)

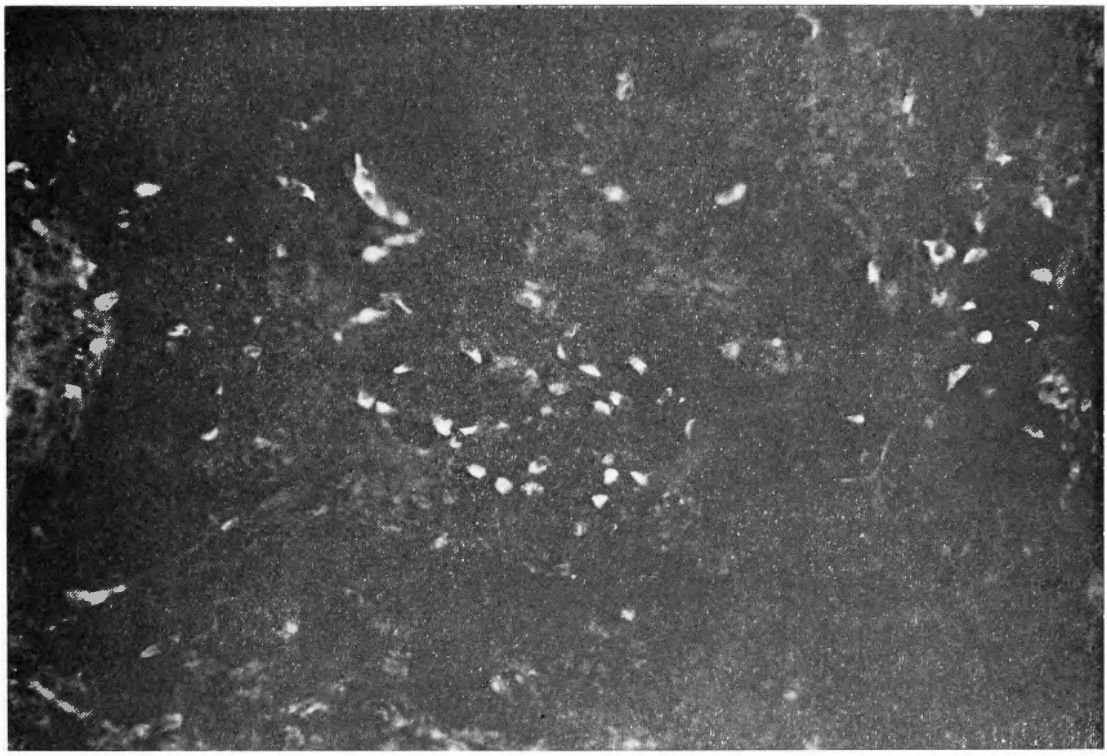

Fig. 3. Gastrin-containing cells in the second portion of the human duodenum (× 160)

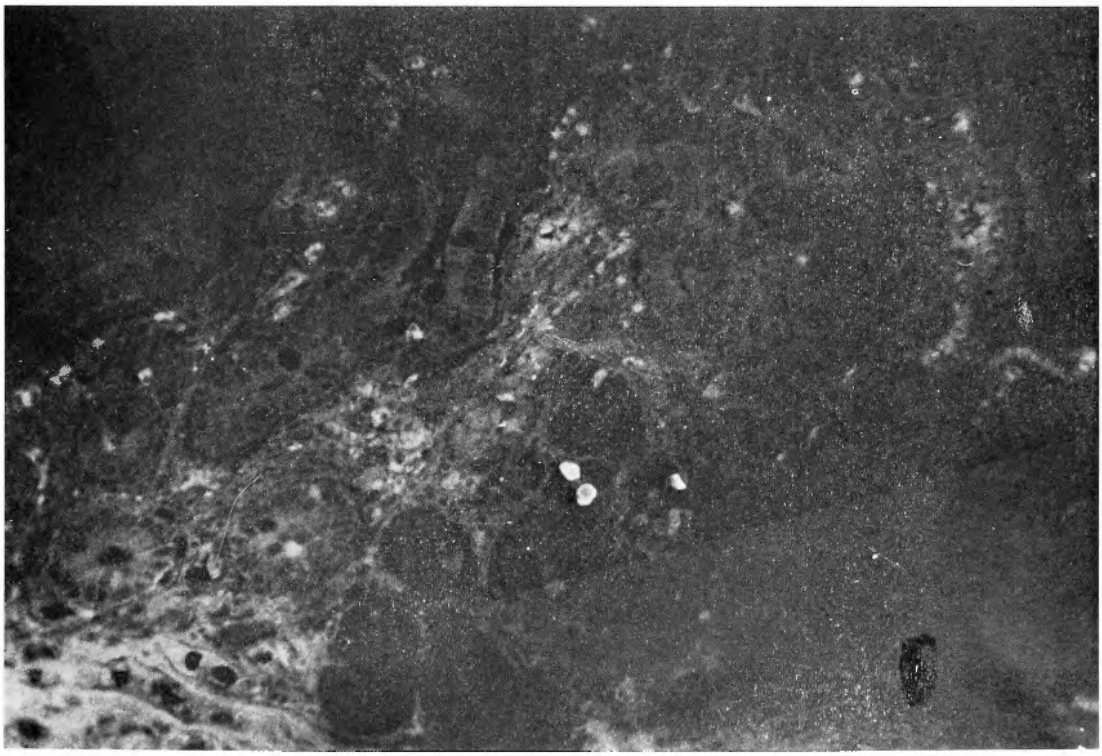

Fig. 4. Gastrin-containing cells in the 3rd portion of the duodenum. (×160) The population is very scant.

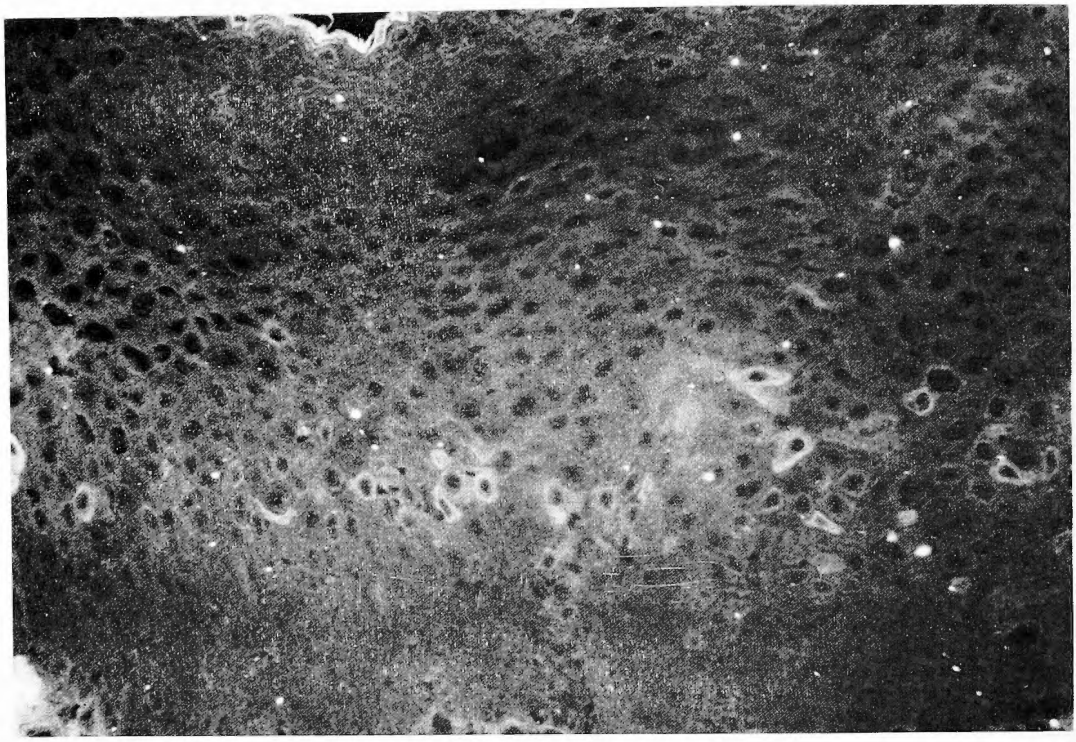

Fig. 5. No gastrn-containing cells can be identified in the jejunuim. (× 160)

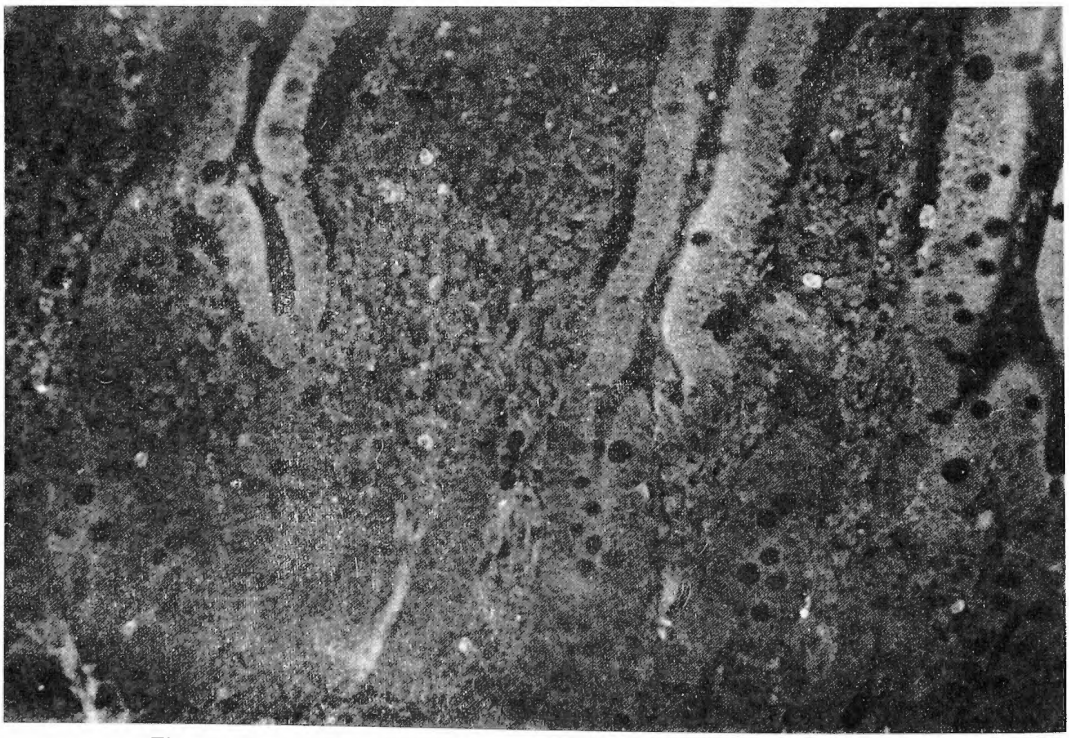

Fig. 6. No gastrin-containing cells can be identified in the ileum. (× 160)

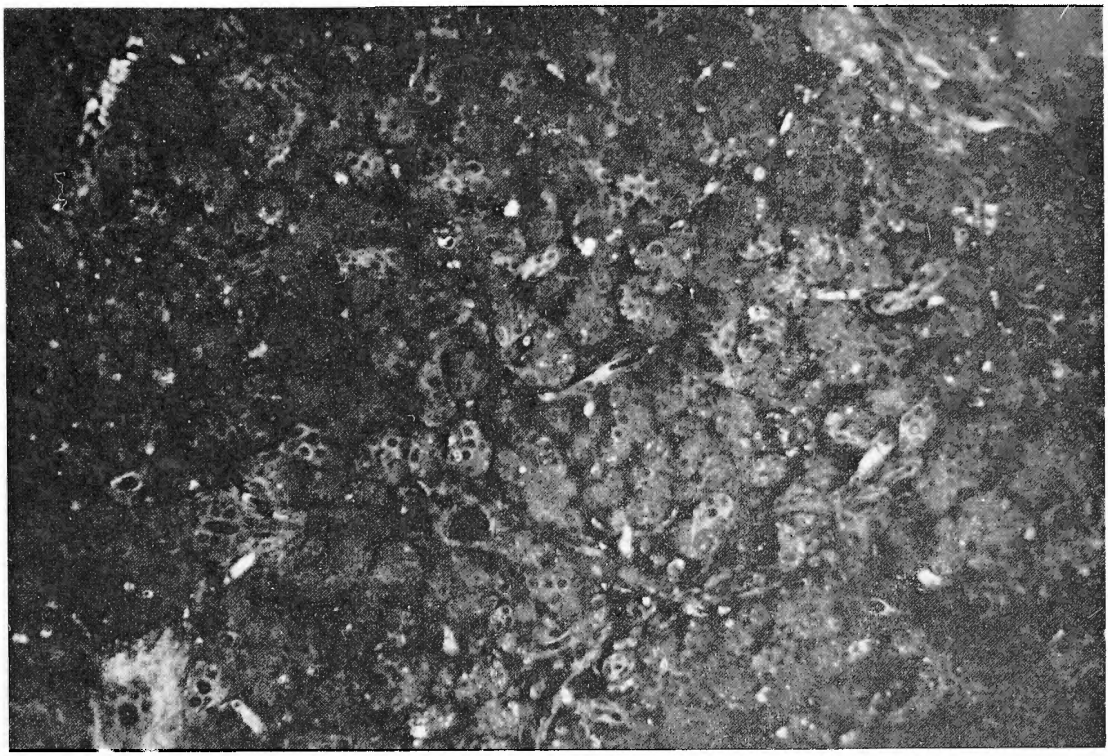

Fig. 7. No gastrin-containing cells can be seen in the human pancreas. (× 160)

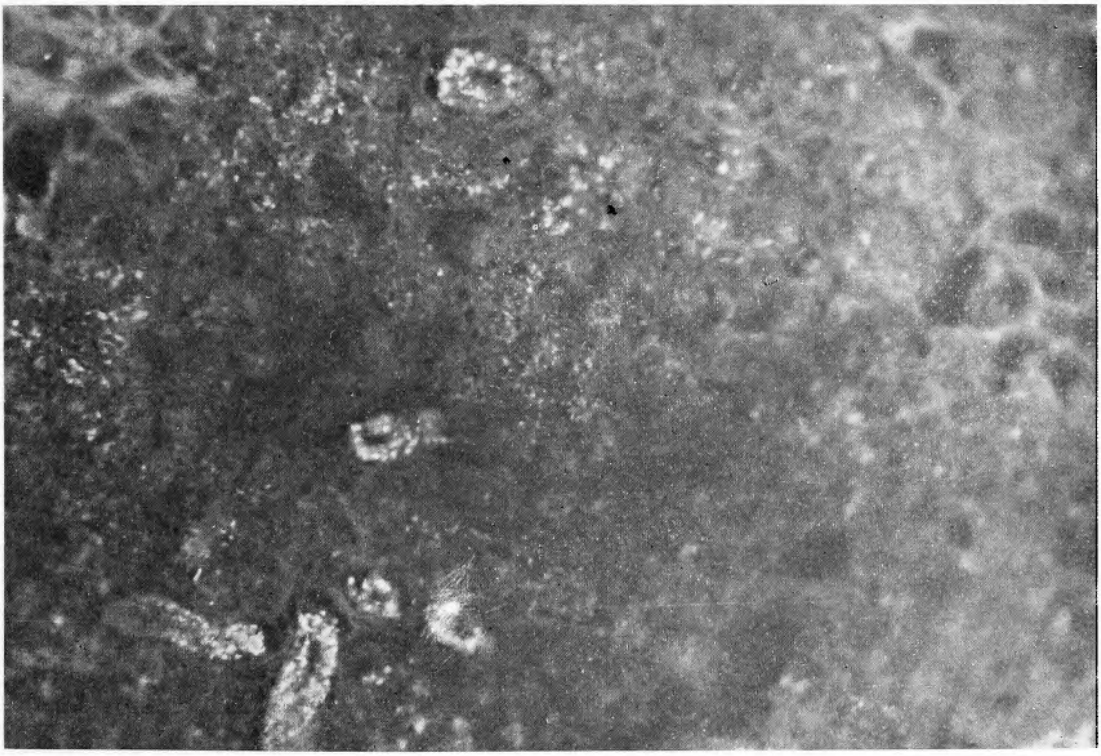

Fig. 8. No gastrin-containing cells can be identified in the parotid gland. (× 160)

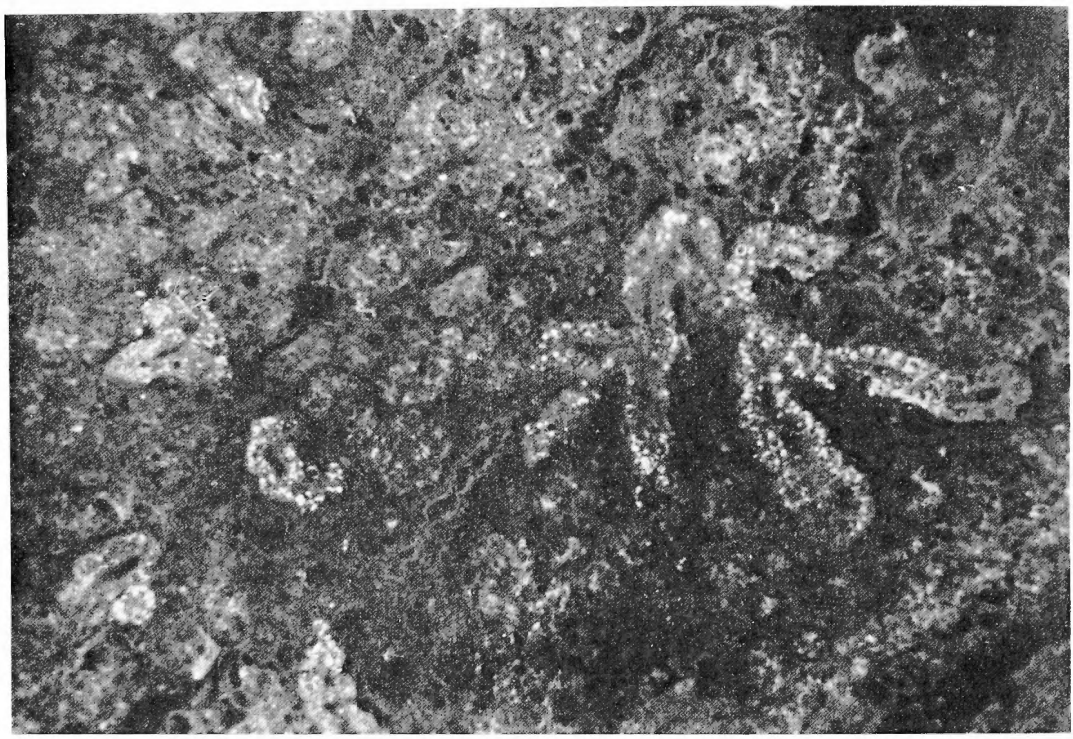

Fig. 9. No gastrin-containing cells can be identified in the submandibular gland. (× 160)

Careful studies were done on fresh specimens of surgically removed pancreas, parotid gland and submandibular gland. Numerous non-specific autofluorescent granules were demonstrated by fluorescent microscopy, however, specific gastrin-containing cells could not be seen in our study (Fig. 7, 8, 9).

These results are summarized in Fig. 10.

Distribution of gastrin-containing cells in canine digestive organs

Stomach :

Gastrin-containing cells were numerous in the antrum of the dog stomach (Fig. 11) as we have already noted in the human stomach. They were dispersed in the cryptal epithelium and situated in the middle zone of the mucosal thickness. They were pyramidal in shape, extending a long process into the gastric lumen.

In the cardia and body (Fig. 12) of the stomach there were no gastrin-containing cells. The margin of the distribution of gastrin between the antrum and the body was sharply defined.

Duodenum :

In the dog duodenum gastrin-containing cells could be found in the duodenal bulb. They were fewer than in the human duodenum. There were no gastrin-containing cells

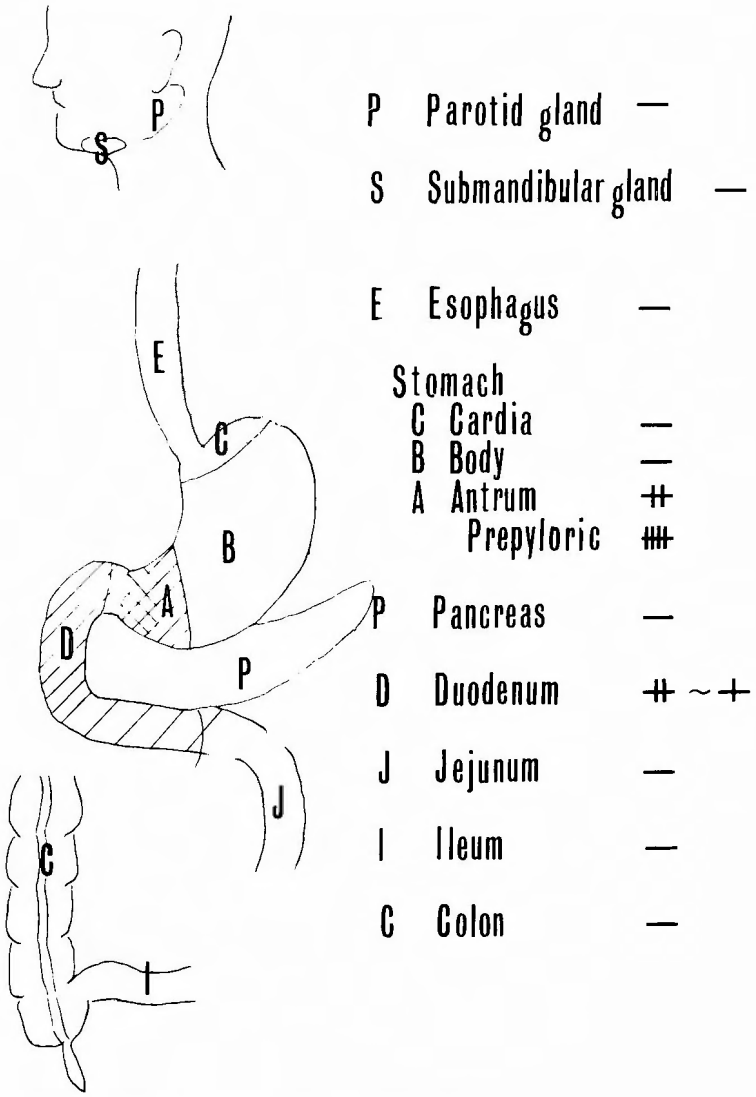

Fig. 10. Distribution of gastrin in human digestive tract demonstrated by direct immunofluorescence.

distal to the papilla of Vater.

Esophagus, jejunum, ileum, colon and rectum :

No gastrin-containing cells could be identified in the esophagus, jejunum, ileum, colon or rectum.

Pancreas and salivary glands

Careful studies were done on fresh specimens of pancreas, parotid gland and submandibular gland. Though numerous areas of non-specific granular autofluorescence were

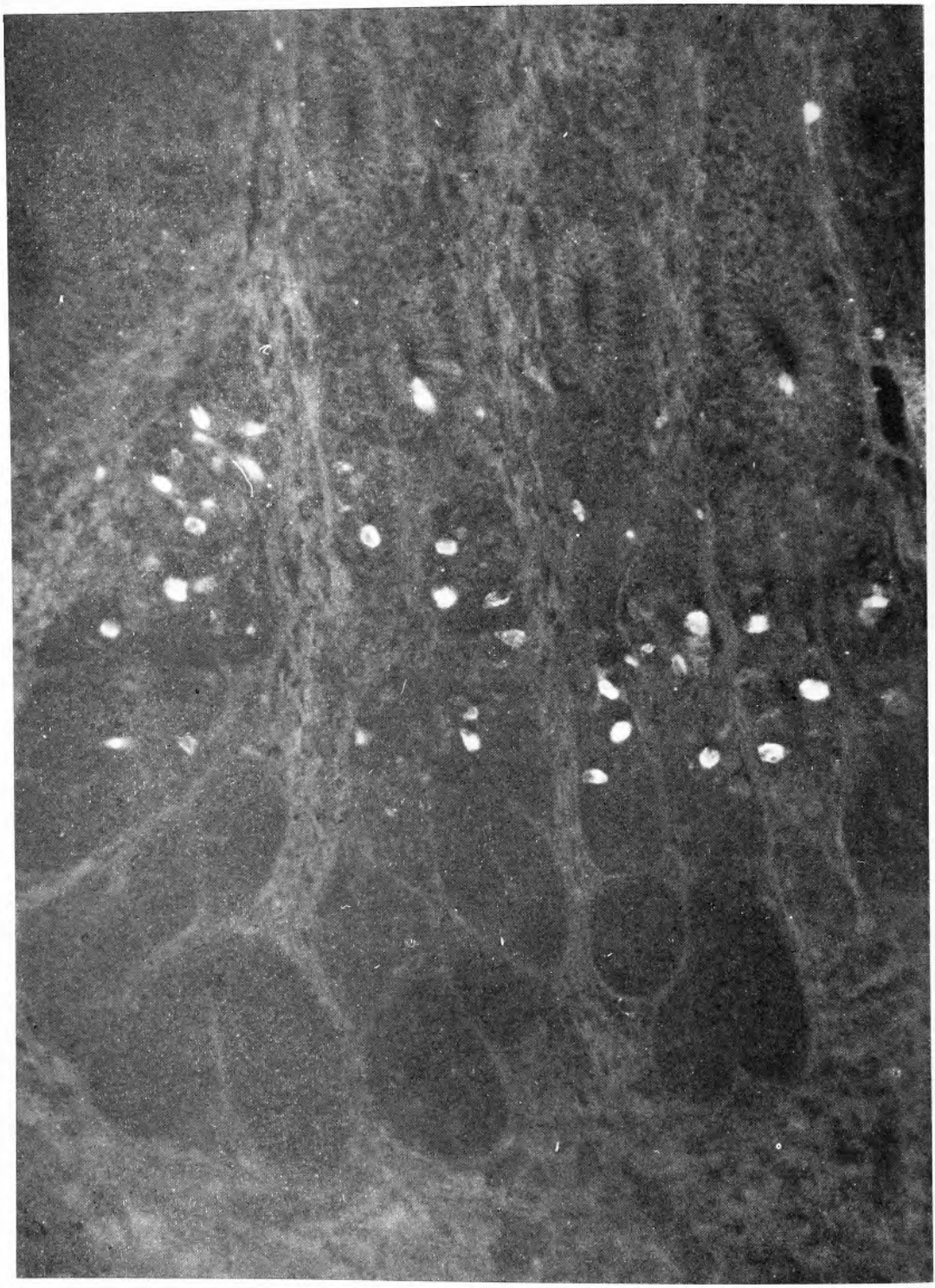

Fig. 11. In the antrum of the dog stomach gastrin-containing cells are numerous. (× 160)

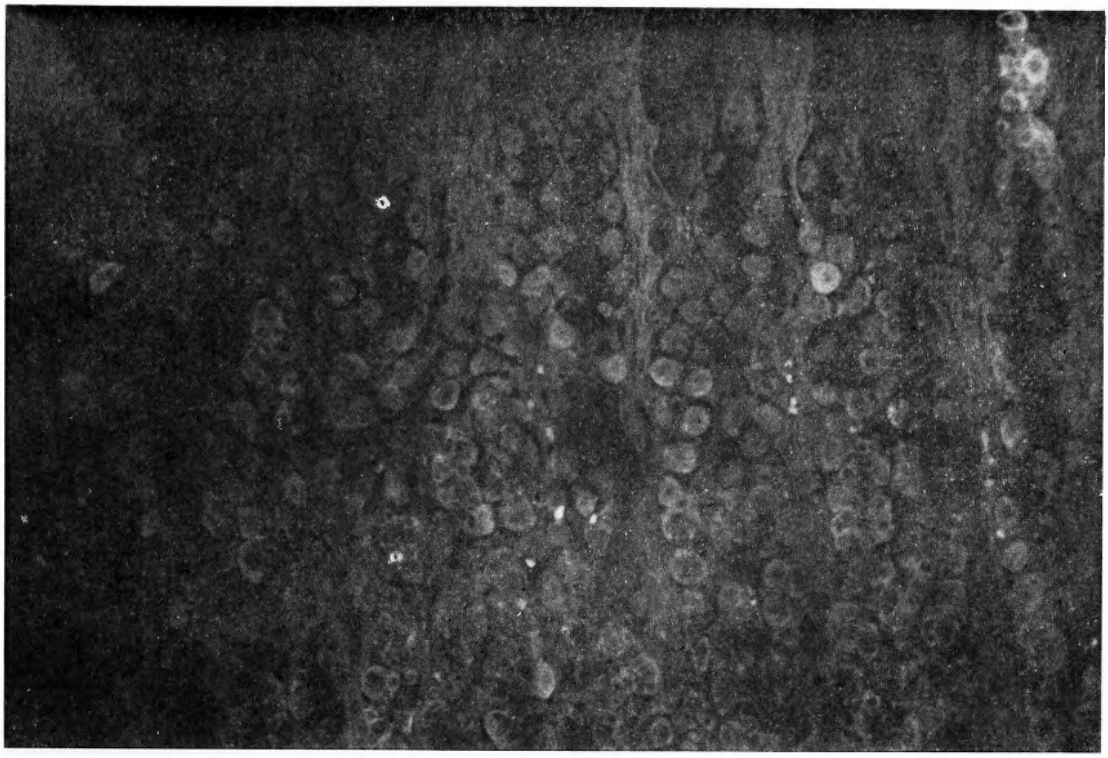

Fig. 12. In the gastric body of the dog there are no gastrin-containing cells.

demonstrated, especially around the duct as in the human, but gastrin-containing cells showing a specific reaction could not be seen in our study.

The distribution of the gastrin-containing cells in the dog digestive tract and glands as revealed by this study was summarized in Fig. 13. This was similar to that in the human digestive organs except in the duodenum, which was scanter in the dog.

Distribution of gastrin-containing cells in cat digestive organs

In the cat digestive organs gastrin-containing cells were distributed in the antrum (Fig. 14) and in the duodenum as in the dog. Distribution in the duodenum was the same as in the dog but fewer than in the human. They were concentrated in the duodenal bulb, and no gastrin-containing cells were seen distal from the papilla of Vater.

These findings were summarized in Fig. 15.

Discussion

Several kinds of polypeptide hormones and biogenic amines in the gastro-entero-panc reatic endocrine system play an important role in the secretions and motility of the mammalian digestive system. Gastrin is one of the most important endocrine hormones in relation to stomach surgery. The role of gastrin in the pathophysiological conditions before and after gastrectomy has gradually been elucidated. The distribution of gastrin in the human gastrointestinal tract has been described by McGuigan et al[12)13)] and by Bussolati et al.[1)18)]

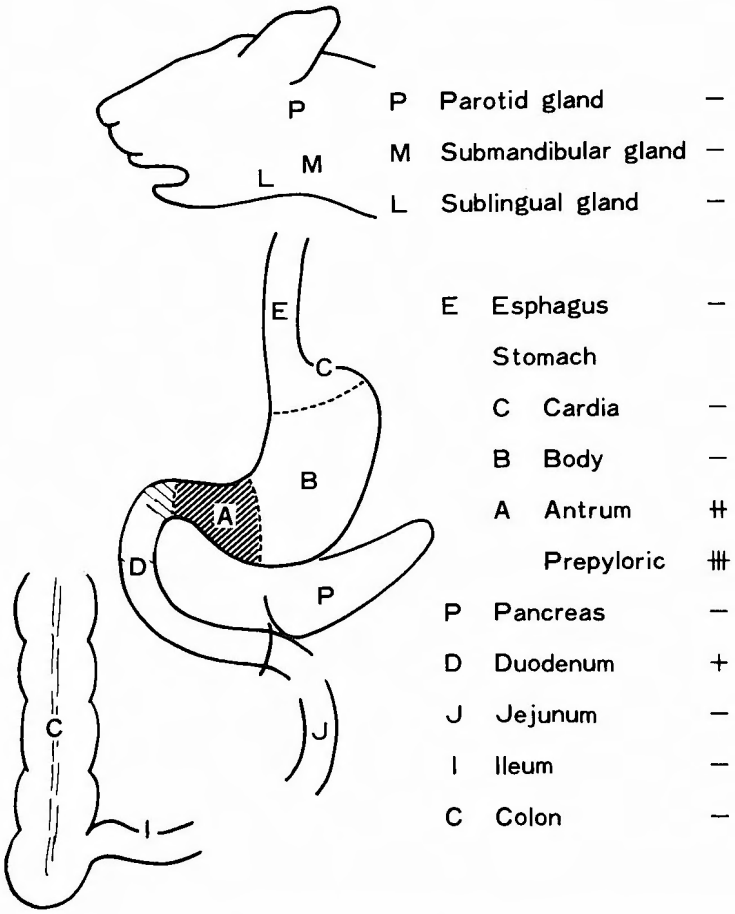

Fig. 13. Distribution of gastrin in canine digestive system demostrated by direct immunofluorescence.

There are three difficult problems in the immunofluorescent demonstration of gastrin: 1) The molecular weight of gastrin is so low (Gastrin I, 2096 ; Gastrin II, 2176) that antigastrin antiserum can be obtained only when synthetic gastrin is conjugated with bovine serum albumin -- a chemical technique. 2) Rabbits can be immunized by bovine serum albumin conjugated with synthetic human gastrin for long periods -- an immunological technique. 3) Because antigastrin antiserum is expensive and scarce, many investigators use an indirect immunofluorescent technique, which causes nonspecific staining. A direct immunofluorescent technique should be used -- an immunohistochemical technique.

In this study a direct immunofluoresent technique was used with FITC labelled IgG fraction of specific anti-SHG I antiserum.

There have been many reports on the distribution of gastrin in human digestive organs using bioassay, radioummunoassay and immunofluorescence, but no report about dog and cat.

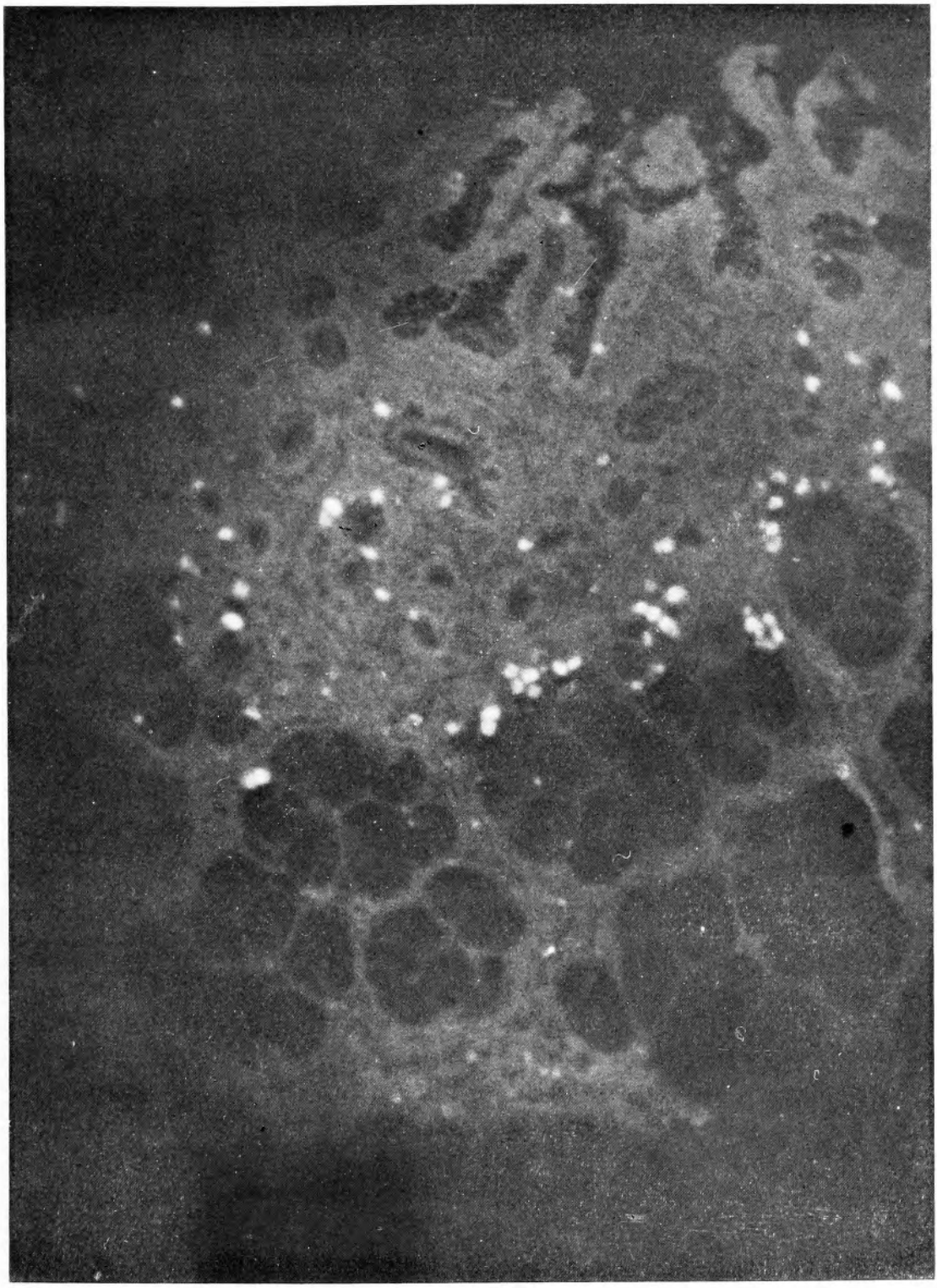

Fig. 14. In the antrum of the cat gastrin-containing cells are demonstrated. (× 160)

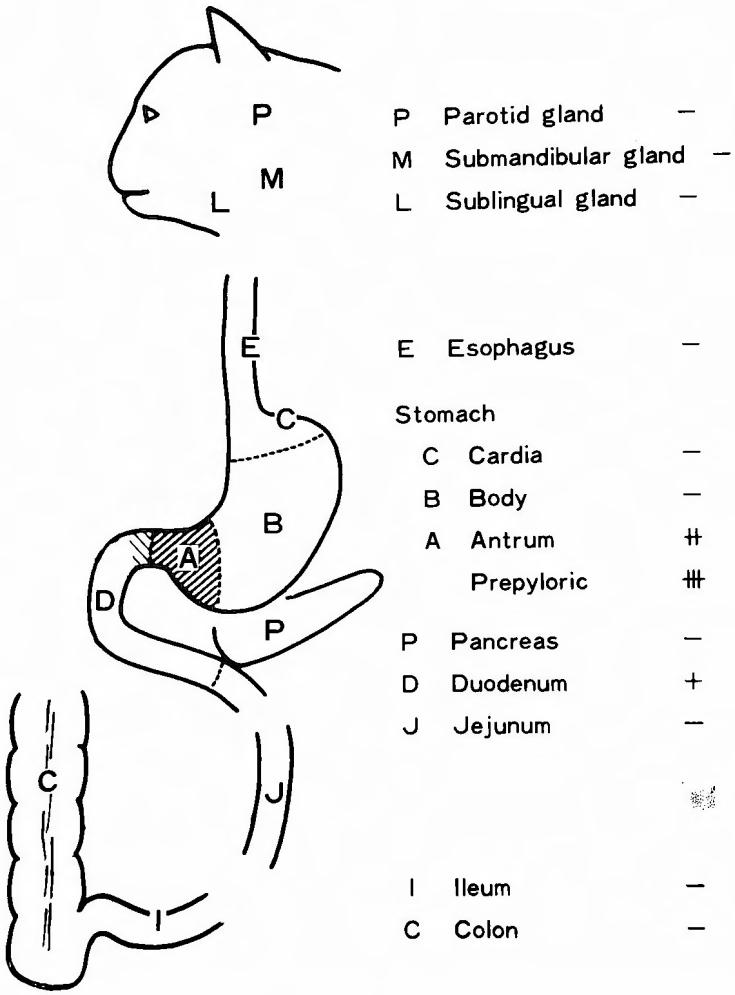

Fig. 15. Distribution of gastrin in cat digestive system demonstrated by direct immunofluorescence.

A highly specific direct immunofluorescent technique showed that gastrin cells are present only in the antrum and duodenum, as showed some differences from those reported by other investigators. The chief points of difference are :

1. Gastrin in the cardia by bioassay[4]. (EDKINS, 1906 ; GREGORY and TRACY 1961)
2. Gastrin in the pancreas (using bioassay, OMOLE, A.A. et al.[17], 1972), (using indirect immunofluorescence. LOMSKY, R.F., LANGER, F. and VORTEL, V.[9] 1969 ; GREIDER, M.H. and MCGUIGAN, J.E,[8] 1971)
3. Gastrin-like immunoreactivity in salivary glands (using radioimmunoassay by TAKEUCHI, T.[22] et al 1973)

The direct immunofluorescent technique is more highly specific for the demonstration of cellular localization, especially for little gastrin, than bioassay or indirect immunofluorescence.

In the human stomach gastrin-containing cells were numerous in the antral mucosa. The shape and location of these cells were as described in other papers[1,12,13,18,23]. The richest distribution was in the antral mucosa, 2-4cm from the pylorus. In the body and cardia no gastrin-containing cells were seen.

In the duodenum some gastrin-containing cells were seen, but fewer than in the stomach and their number decreased distally. In the jejunum no gastrin cells were seen.

There are conflicting opinions about the existence of gastrin-containing cells in the pancreas. In the Zollinger Ellison syndrome, non B-cell islet tumor produces gastrin or a gastrin-like substance. OMOLE and AMURSE et al[17] demonstrated gastrin activity in the mammalian pancreas. MCGUIGAN et al[1,12,13,18] using immunofluorescence, reported the presence of gastrin-containing cells. LOMSKY et al[9] using an indirect immunofluorescent technique, also reported gastrin in mammalian islet cells, which they believed to be D-cells.

In the pancreas numerous autofluorescent granules can be seen by fluorescent microscopy, but these can be eliminated by a blocking test.

The specific direct immunofluorescent technique demonstrated no gastrin-containing cells in many fresh specimens removed at laparotomy in our study. The international symposium[20] held in Bologna in March, 1973 (Revised Wiesbaden classification of gut endocrine cells) agree with our results.

In the parotid and submandibular glands immunoreactive gastrin and immunofluorescence for gastrin were reported by TAKEUCHI et al. In these salivary glands the same autofluorescent granules as noted in the pancreas were numerous but could be eliminated by the test. However, no gastrin-containing cells could be seen. This finding was also confirmed in dog salivary glands. No gastrin-containing cells were seen in the esophagus, or intestines. From these results serum gastrin levels in pathophysiological condition after surgical operation could be understood theoretically.

Pancreaticoduodenectomy which remove antrum and duodenum with pancreas head (Fig. 16) resulted in complete gastrin defficiency -- that is iatrogenic agastrinema -- and radioimmunoassay in this condition shows no gastrin response. (This fact was confirmed by FUKUCHI[7] in our laboratory.)

Gastrin levels after gastrectomy showed interesting results. Cancer patients after removal of the distal portion of the stomach and gastrojejunostomy (in this route no gastrin containing cells distribute) showed little or no response to oral glycine solution. However B_1 type gastroduodenostomy showed response (Fig. 17).

Recent studies by WATSON L.C. et al[24] using radioimmunoassay showed gastrin concentrations in the upper gastrointestinal mucosa in dogs, with the major source in the antral mucosa; only 4% of the total mucosal gastrin was extracted from the fundus and small bowel. They concluded that extra-antral sites, that is extra-gastric gastrin in the dog, do

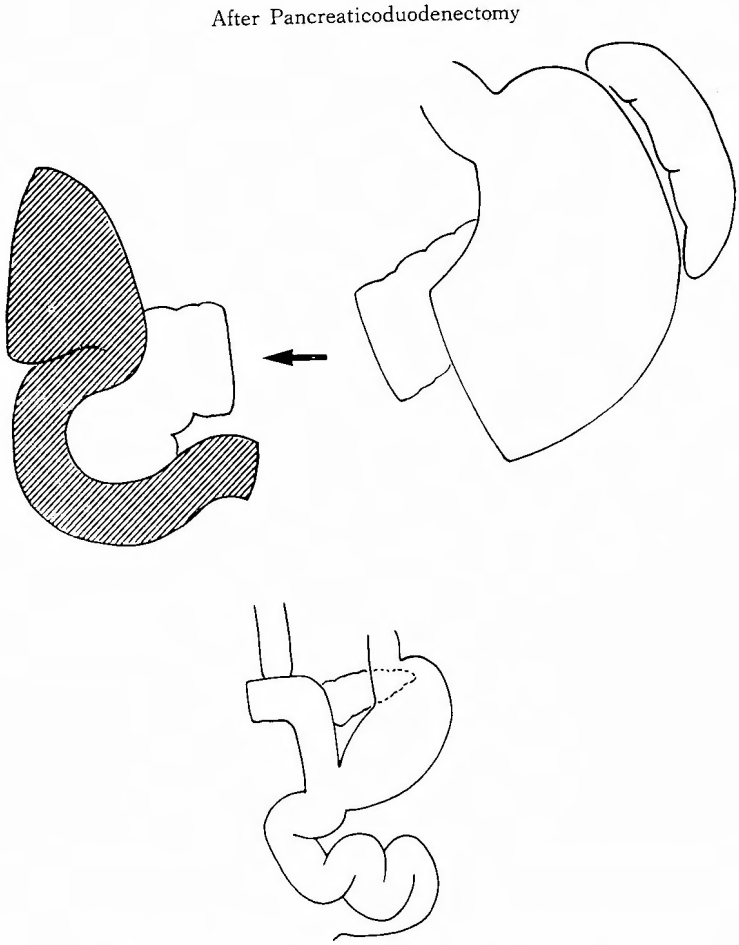

Fig. 16. The figure shows that there are no gastrin-containing area after pancreaticoduodenectomy.

not contain as great a concentration of gastrin as has been reported in man.

Morphologically, however, G-cells can be found by electron microscope in the dog duodenum. An immnofluorescent technique (specific direct method) can also demonstrate gastrin-containing cells in the duodenal bulb.

Because dogs and cats are usually used as experimental animals for gastrin release by vagal or local (pH) stimulation, the distribution of gastrin in the duodenum in relation to the vagal nerve is an important problem. Gastrin-Canaitning cells in the dog and cat duodenum is distributed mainly in the area of the hepatic branch of the anterior vagus.

The relationship between gastrin-like immunoreactivity substance, which can be determined by radioimmunoassay, and heptadecapeptide molecular form gastrin which can be demonstrated by immunofluorescence or electron microscope is an interesting problem to consider.

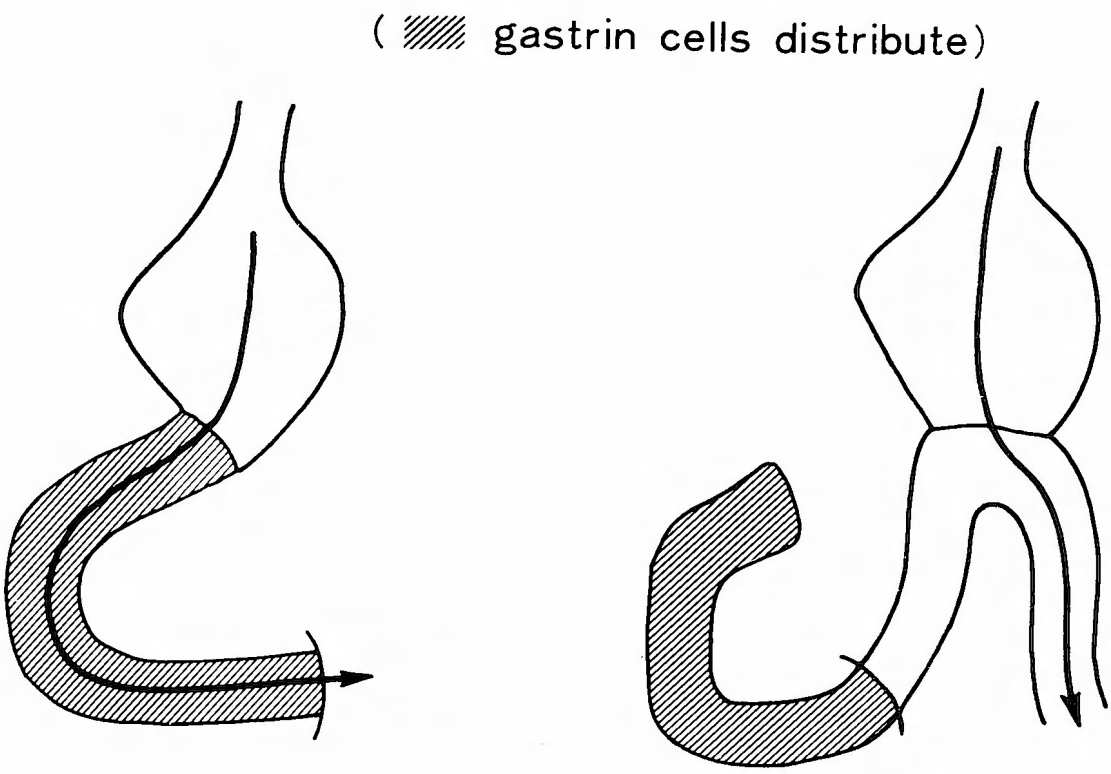

Fig. 17. The figure shows that there are two routes of diet after gastrectomy in relation to gastrin distribution.

Summary and conclusion

A highly specific direct immunofluorescent technique was used to determine the distribution of gastrin-containing cells in the digestive system of human, dogs and cats, and to compare it among three species.

In human, dogs and cats, gastrin-containing cells were found only in the antrum and in the duodenum. The distribution within the duodenum, however, differed in humans from that in dogs and cats.

In the human duodenum gastrin-containing cells were seen in all portions from the pylorus to the 3rd portion, in gradually decreasing concentration, while in the jejunum no gastrin-containing cells were seen. However in the dog and cat duodenum gastrin-containing cells were found only in the duodenal bulb, and none were seen distal to papilla of Vater.

No gastrin-containing cells were seen in the esophagus, cardia, body of the stomach, jejunum, ileum, colon, pancreas, parotid gland or submandibular gland in humans, dogs and cats.

The limits of distribution between the antrum and body of the stomach were sharply demarcated in all three species.

From these results, the change of serum gastrin levels in pathophysiological condition after gastrointestinal surgical operation could be understood theoretically.

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Some of these figures were published in Arch. Histol. Jap.[23]

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和文抄録

ヒト，イヌ，ネコ消化管におけるガストリンの分布（螢光抗体法直接法による検索）

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ガストリンは胃液分泌胃相の中心を担う消化管ホルモンであり，1964年その構造がGregory等により決定され次いで1968年 McGuigan によりその抗体が作成され，放射免疫検定（RIA）が開発され血中ガストリンの測定が可能となり消化器外科の分野でも，胃十二指腸疾患の術前術後の病態生理の解明に広く用いられるようになった．一方ガストリンの分布は主としてBioassay が中心であったが，ガストリンの抗血清が作成されて以来螢光抗体法，RIA により検索が始められたが，諸家により，その結果がまちまちである．著者らは純品のヒトガストリンⅠを抗原として抗血清を作成し，螢光抗体法直接法により，ヒトガストリンの分布を検索し，更にその交叉反応を利用して，イヌ，ネコのガストリン分布を検索．RIA にて胃切除手術の術前術後のガストリン値を測定し，分布の妥当性を再確認した．結果

(1)ガストリンを含む細胞は胃幽門前庭部の特に幽門部から2～4㎝までの部分の粘膜に最も豊富に存在する．胃体部の噴門部には見られない．

(2)胃以外では，ガストリン保有細胞は十二指腸のみに存在するが，その数は少なく，又十二指腸でも遠位になる程その分布は粗となる．

(3)食道，空腸，回腸，結腸以下の大腸には，ガストリン保有細胞は存在しない．

(4)膵には自家螢光が多く見られるが，ガストリン保有細胞は存在しない．耳下腺，顎下腺などの唾液腺も膵と全く同様である．

(5)イヌ，ネコにおけるガストリン保有細胞の分布もヒトとほぼ同様である．ただ十二指腸における分布が少し狭くなる．